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Morphogenesis of Digestive Tract of *Bufo melanostictus* Schneider.

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The process of differentiation, growth and metamorphosis of amphibian digestive tract is very complex and interesting. Since second half of the nineteenth century various workers have studied the various aspects of this problem in different amphibians. Gotte (1875), K. Pressler, (1911) have studied it in *Bombinator igneous;* Reuter (1900) and Duesberg (1906) in *Alytes obstetricans;* P. Joly (1937) in *Discoglossus pictus;* Balinsky (1956) in *Xenopus laevis;* Bower (1909) in *Bufo lentiginosus;* and quite recently Nakamura & Tahara (1953, 1954,) in *Rana nigromaculata* and Albert (unpublished) in *Rana dalmatina*.

Present study has been taken with a view to study the stage by stage morphoge-, nesis of digestive tract of *Bufo melanostictus* starting from the undifferentiated, heavily yolked endodermal cellular mass to the fully metamorphosed digestive tube. The stages employed during tis study are described by the author in the Normal Table of *Bufo melanostictus* (Khan 1965). No similar work has been done on this species.

Material and Method

The material for this study was collected from nature. It has been found out that the development of the stages from 26th to 37th (Khan op. cit.) does not proceed normally in the laboratory conditions. The tadpoles so reared show a greater ratio of mortality, moreover their digestive tract remains underdeveloped and abnormal as compared to those collected from nature. For this reason, after a downpour, natural breeding sites of *Bufo melanostictus* were marked, and at regular intervals the material was collected from these areas.

It has also been observed that the tadpoles of different hatches, even of the same hatch, differ in the extent of the development of their digestive tracts. Hence care has been taken to store separately the tadpoles of different hatches. This study is based on the tadpoles of a same hatch, though frequent comparison has been made with the tadpoles of other hatches belonging to the same stage. To ensure precision the different configurations of digestive tract are recorded up to the Stage 25, because the later stages do not show much disparity. For this reason Stages from 21 to 25 showing disparity are divided into early and late Stages for convenience sake.

Five to eight tadpoles belonging to a Stage were dissected to ensure the record of more normal and natural configuration of the digestive tract at that stage. The dissection dish was made by pouring molten wax in a large petri dish so as to fill it about half of it and allowing it to cool. A narrow groove about ½ inches in breadth and 2 inches in length was made in the center of this dish. The tadpoles were held by a pin passing through the tail from side to side, then the pin was inserted in the wall of the groove so that the ventral side was facing upward. The more or less transparent abdominal wall was incised by

fine scissors. To study the intestinal coils the intestinal spiral was lifted gently with the help of two fine needles or forceps and it was pinned down along one side of tadpole.

Tadpoles were fixed in Bouin's fluid anp stored in diluted Bouin's fluid as already suggested (Khan op. cit.). For study, thus stored material was transferred in a mixture of 70 % alcohol : 1 part; Glycerine: ½ parts, so to avoid the brittleness of the material during study.

Drawings were made by the help of Camera lucida.

DESCRIPTION

The development of digestive tract, in *Bufo melanostictus*, for convenience sake, could easily be divided into three distinct periods.

(*i*) *The period of Pre-larval differentiation of the endodermal Mass* into a prototype of digestive tract (Stages 21-23).

(*ii*) *The period of development of larval digestive tract* into a distinct dorsal (snistral) and ventral (dextral) coils intertwined with each other. (Stages 24-37).

(*iii*) *The period of post-larval regression and differentiation of the spirally entwined digestive tract* into a more simple, short and regionally differentiated adult digestive tract (Metamorphosis, Stages 38-43).

Period of Pre-Larval differentiation of the endodermal mass:

The endoderm, destined to form the larval digestive tract, is in the form of a compact, heavily yolked cellular mass. At Stage 17 it is *latero*-laterally compressed breadth being constant from anterior to much of the posterior part which tapers to end. Stages 18, 19 & 20 show a gradual, amassing of the endodermal material in the anterior half, so to give the endodermal mass a pyriform appearance which is cylindrical in outline and tapers posteriorly.

g 21 (Fig 1,2)

Early:

The endodermal mass measures to 2 mm in length. It is pear shaped; rostrally broadly rounded and gradually tapering

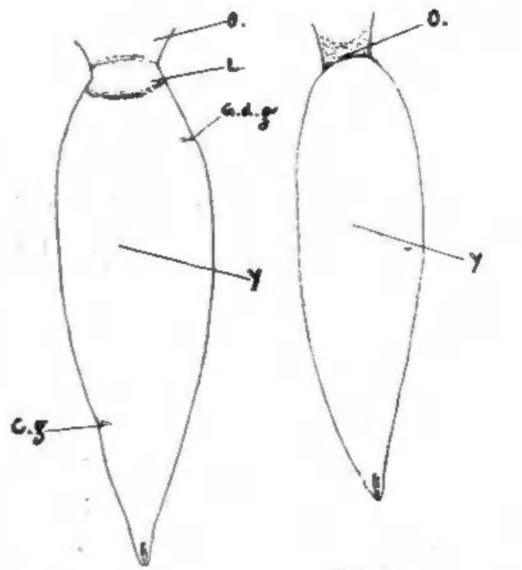

FIG. 2 Stage late 21 x 70

FIG. 1 Stage early 21 x 70

Figs. 1 and 2. Ventral view of digestive tract at early and late Stage 21.

caudad. The caudad tapering part forms an angle of 60° with the tail. The endodermal mass is compact and does not show any sign of distortion etc. The oesophagus is massive and cylindrical, it joins the endodermal mass along dorsal side.

Late:

A slight caudad elongation in the posterior narrow part of the endodermal mass is noted. The endodermal mass measures 2.2 mm in length.

On dissection two horizontal grooves along right and left sides of the endodermal mass could be seen. The anterior left one third part of the endodermal mass shows a slight groove directed towards right of the embryo marking the *rostral* or *gastroduodenal groove*. Similarly another groove, the *dorso-ventral* or *caudal groove* is discernible just anterior to the caudadly lengthened part along the right side of endodermal mass.

A slight bulge in the anterior, median ventral part of the endodermal mass, marks the appearance of the liver analage.

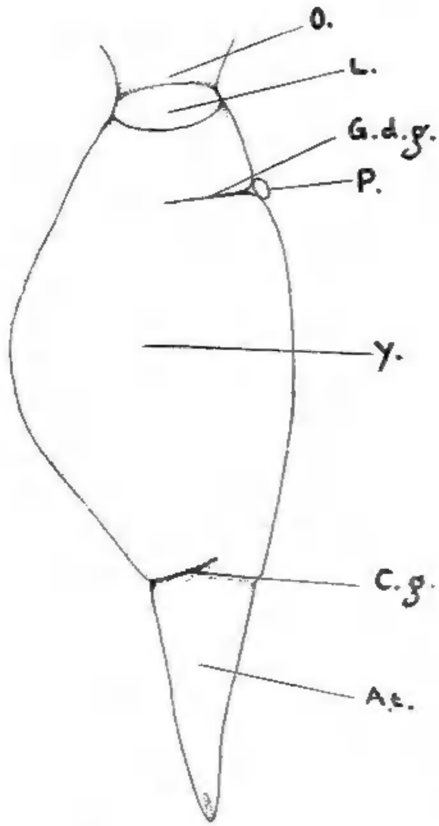

FIG.3 Stage 22 x70

Fig. 3. Ventral view of digestive tract at Stage 22.

Stage 22 (Fig 3)

Rostral and dorso-ventral grooves deepen in the endodermal mass and run horizontally towards right and left sides of the embryo, respectively. Due to these two opposite growing grooves, the main bulk, lying between, bulges along the right mesial side of the endodermal mass.

Along the left ventral side of the dorsal groove a roundish structure, the pancreas makes its appearance.

The liver analage is quite marked.

These structures are visible at this stage, through the transparent abdominal wall. The endodermal mass measures 2.5 mm from the oesophagus to the posterior end

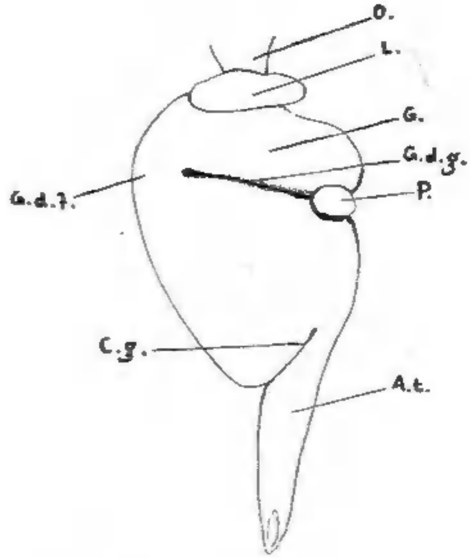

FIG. 4 Stage early 23 x70

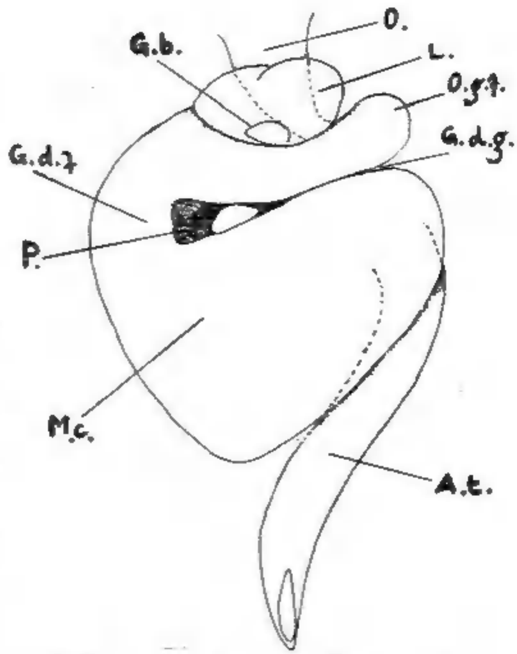

FIG.5 Stage late 23 x70

Figs. 4 and 5. Ventral view of digestive tract at early and late Stage 23.

of the endodermal mass. The caudad lengthened part is quite marked and becomes more tubular.

Stage 23 (Fig 4&5)

Early:

The mesial bulge observed at Stage 22 along the right side, shifts rostrally forming a curve round the right end of the rostral groove, thus demarcating the anterior horizontal limb (the future stomach), from the main endodermal mass. The horizontal limb, thus resulted, is continuous with the main endodermal mass through the *gastro-duodenal flexion* (the bulge at right side observed earlier).

Meanwhile, the caudal furrow has cut off a tubular portion from left side of the endodermal mass, which is continuous posteriorly with the posterior lengthened part of the endodermal mass. This long tubular limb partially lies on the dorsal side of the main endodermal mass and becomes continuous with it through a flexion, the *ilio-rectal flexion.*

The oesophagus runs obliquely towards posterior left side and curves sharply round the left side of the liver towards ventral right side to become continuous with the gastric limb of the endodermal mass thus forming *oesophagio-gastric flexion.*

The main endodermal material is heart-shaped with the broad rostral face and caudad pointed end.

Late:

The various flexins are much accentuated and the endodermal mass of Stage 21 can be made out into a zigzag prototype of digestive tract which could be the distinguished into the following four regions.

(*i*) Obliquely situated, rostro-caudad directed *oesophagio-gastric part* forming the *oesophagio-gastric flexion.*

(*ii*) Horizontal, *gastric region* continuous with the oesophagio-gastric flexion along the left and the main endodermal mass along the right side forming the *gastro-duodenal flexion.* It is flexed itself to form a crecentric "*gastric loop*".

(*iii*) Crecentric *main lobe* continuous with the gastro-duodenal flexin on the left and forming *ilio-rectal flexion* on right. This is the broadest part of the developing digestive tract and is heart-shaped.

(*iv*) Caudorostrally directed *rectal part* (anal tube) continuous with ilio-rectal flexion lying on the left side of the main lobe. This is tubular opening to exterior at the cloacal aperture which is quite large and oval.

The liver is bi-lobed and thin leaf-like, abutting against the gastric loop rostrally. The gall bladder is quite large and oval It is distended and has very thin walls. It lies along the dorsal side of the right lobe.

Pancreas occupies the gastro-duodenal loop. The intestine measures 3 mm from oesophagus to the cloacal aperture.

The period of development of larval digestive tract :

Stage 23 marks the development of a prototype of the future digestive tract. The point for the differentiation of the ventral and dorsal coils of the larval intestinal spirals is established during the 24th Stage and this process of coiling goes on until Stage 37.

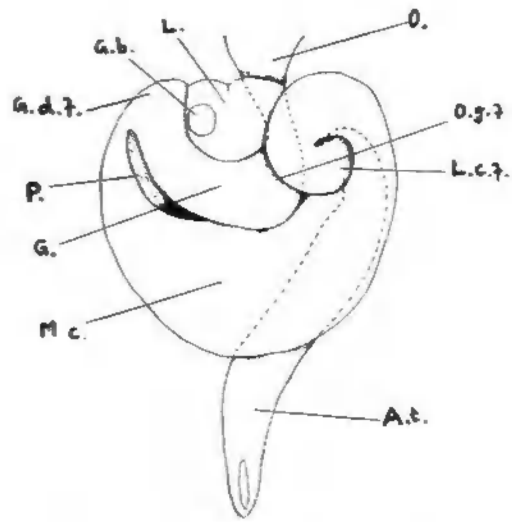

FIG. 6 Stage early 24 × 70

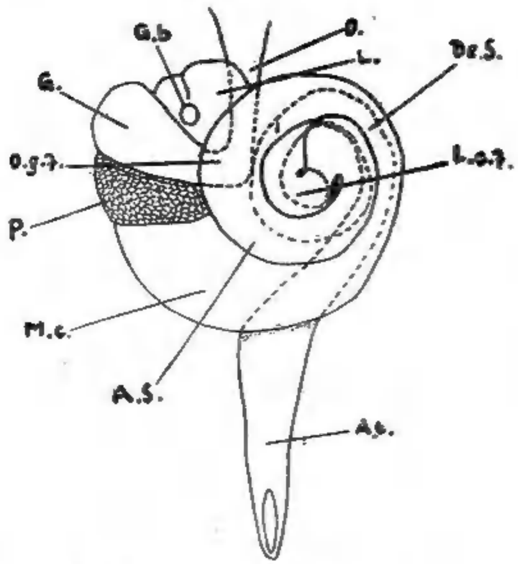

FIG.7 Stage Late 24 x70.

Figs. 6 and 7. Ventral view of digestive tract at early and late Stage 24.

Stage 24 (Fig (9 7)

Early:

The gastric loop is most accentuated enclosing the liver. The main lobe is more cylindrical and narrow, in future it will be referred to as the *main coil.* Its right and left ends abut against the branchial cavity of the both sides. The left end after abutting against the left branchial cavity curves round towards right and turns caudad to curve again towards the left side, thus forming a very close U-shaped coil. The one end of the coil is continuous with the main coil other with the posterior part—rectal part, through ilio-rectal flexion. This coil marks the point of coil-differentiation, in future it will be referred to as the "*loop of coil formation*" or "point of retrogression" (Albert, unpublished)

Late:

Gastric coil shows a marked decrease in its diameter and acquires more tubular form.

At this stage the spiral entwining of the endodermal material begins to take place. The first coil of the ascending and descending spiral is laid, on the respective ventral and dorsal sides of the "*loop of coil formation*". However, some individuals belonging to this stage show one and a half coil in each spiral. The ascending spiral is anti-clockwise while descending being clockwise.

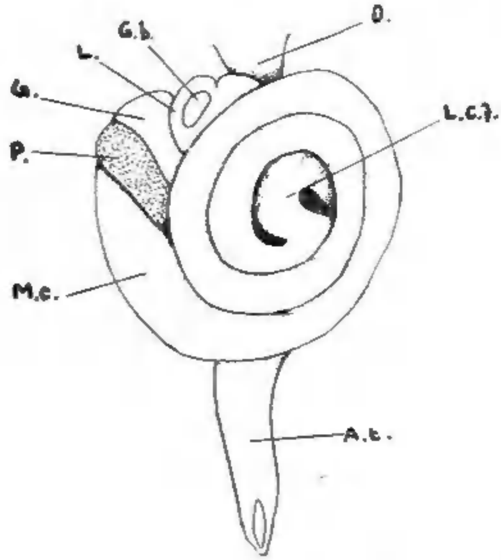

FIG.8 Stage early 25 x70

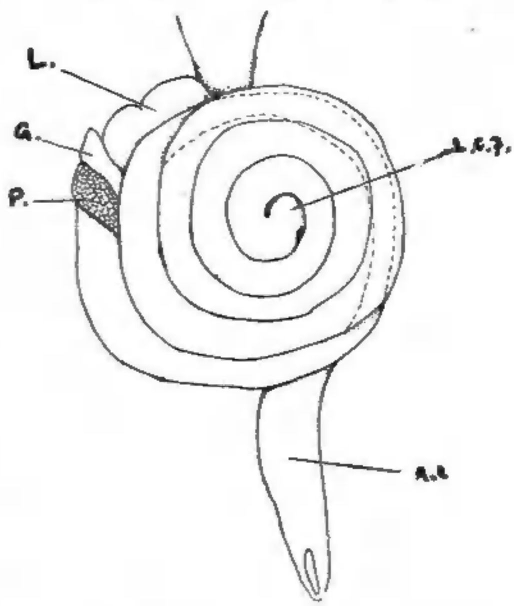

FIG.9 Stage Late 25 x70.

Figs. 8 and 9. Ventral view of digestive tract at early and late Stage 25.

There is a marked decrease in the diameter in the left half of the main coil, as its material is being used for the coil formation of ascending and descending spirals. Right half of the main coil is still broad and massive.

The various flexions show an accentuation in their gyres.

Stage 35 (Fig 8 & 9)

Early:

The ascending and descending spirals show an increase in the number of their coils, as they comprise of two, and one and a half coil, respectively at this stage. There is corresponding decrease in the right half of the main coil. The *gastro-duodenal flexion* is very largely gyred enclosing the liver. The right half of the main coil shows a slight lateral displacement marking the future duodenum.

The *gastric loop*, duodenum, pancreas and liver are being displaced progressively towards left rostral side by the developing spirals.

Late:

Ascending and descending spirals consist of three and a half, and three coils respectively. The gastric region could be distinguished into three parts.

——*Proximal part* (cardia) dorso-ventrally directed rostro-caudad, lying along the saggital plane of larva, continuous rostrally with oesophagus.

——*Middle part*, transversely placed, directed left to right, ventral, forming the *gastric loop*, perpendicular to the saggital plane of larva:

——*Distal part* (Pyloric) ventro-dorsally orientated, forming the *gastro-duodenal flexion*, enclosing the pancreas in its gyre.

The diameter of the distal part of the gastric region is markedly narrower than the rest of the main coil. The main coil has at this stage the same diameter as the rest of the coils.

Rest of the digestive tract can be distinguished into three parts.

——*Main coil* or *principal intestinal loop*, forming the right posterior half of the ascending spiral. Its left anterior half is overlapped by the second coil of the ascending spiral. Its anterior right part is destined to form the duodenum.

——*Small intestine proper*, consists of ascending and descending spirals of larval intestine, entwined anticlock-wise (ascending) and clockwise (descending) with the "*loop of coil formation*" in between. The intestinal spirals are slightly displaced towards left from the middle of the abdominal cavity.

——*Large intestine and rectum*, formed of the last coil of the descending spiral (rectal coil) and the anal tube running obliquely along dorso-ventral plane of the embryo, making an angle of 60° with the tail. It opens to the exterior through the cloacal aperture which is oval and large, situated along the mesial ventral line.

The bile duct could easily be made out. The gall bladder is quite large and distended

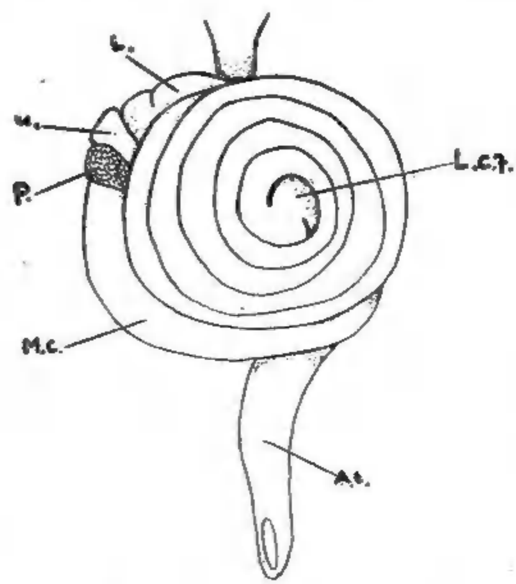

FIG. 10 Stage 33 x 70

Fig. 10. Ventral view of digestive tract at Stage 33.

lying along dorsal side of the right lobe of liver. Pancreatic duct opens in the distal part of the bile duct thus forming a very short hepato-pancreatic duct, which opens in the anterior part of the duodenal region, just after its union with the pyloric part of the stomach.

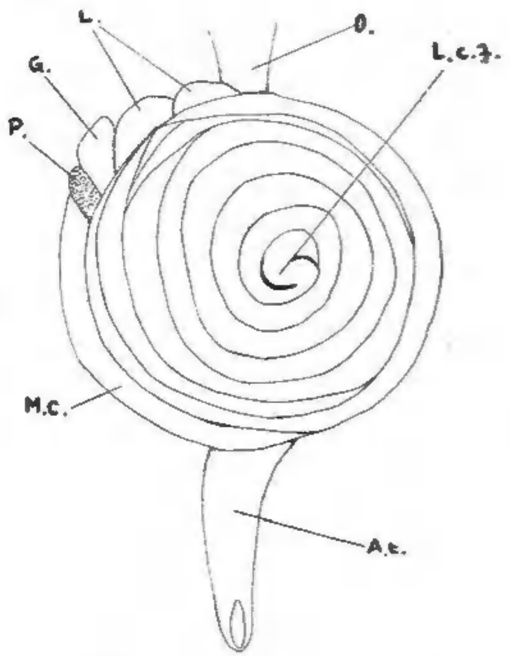

FIG. 11 Stage 37 ×70

Fig. 11. Ventral view of digestive tract at Stage 37.

Stage 26-36 (Fig 10 & 11)

During this period the intestinal coils increase in number and gyre (Table No. 1). The first coil of the descending spiral being the largest, rest of the coils lie one upon another decreasing progessively in size, like a pile of coins. The top of the pile being occupied by the "*loop of coil formation*". The coils of both spirals superimpose each other.

Two last coils of the descending spiral are entwined very sharply and are quite small. The last coil—the rectal coil, continues with the anal tube. The anal tube and the rectal coil show transverse folds along the both sides of their length.

The rapid increase in the number and size of the coils deflects the gastric part, liver, pancreas and the duodenum away from the median line towards left. *Oesophago-gastric flexion* forms an angle of 60°. Only the right end of the gastric part is visible from ventral side, rest of it being overlapped by the intestinal spiral. The increase in number and size of coils involves a corresponding decrease in the diameter of intestine.

There is a gradual shrinkage in the gastric part, especially in its middle region. It becomes thicker than the rest of the intestine except the pyloric part which remains thinner than the rest of the intestinal spirals.

The liver progressively becomes thicker and larger, the gall bladder becomes progressively round in shape but still lying along the dorsal side of the posterior middle part of the right lobe. The bile duct after issuing from the dorsal side of gall bladder runs obliquely and joins the pancreatic duct to form the hepato-pancreatic duct which opens in the right end of the main coil marking the future duodenum.

The tadpole becomes vigorous feeder. It is seen eating most of the time and resting a little. As it feeds large lumps of faecal matter are seen coming out of the anal aperture by the rapid sideway movements of the tail.

The period of post-larval regression and differentiation of the spirally entwined digestive tract:—Metamorphosis.

Stage 37 marks the end of the coil formation. This process though rapid when initiated becomes gradual after 24th stage. Stage 38 marks the initiation of the metamorphosis and differentiation into adult digestive tract. This process is quite rapid and sudden. The tadpole during this period becomes lazy and stops feeding, resting most of the time. This period lasts until Stage 43 which marks the cessation of the process of metamorphosis and the digestive tract at this stage is fully metamorphosed.

TABLE No. 1

Stage	Number of coils: Ascending spiral	Number of coils: Descending spiral	Length from oesophagus to cloacal aperture
21	..	..	2.0 mm
Late	..	..	2.2 mm
22	..	..	2.5 mm
23	..	..	3.0 mm
24	$1, 1\frac{1}{2}$	$1, 1\frac{1}{2}$	0.5, 0.45 cm
25	$2, 2\frac{1}{2}$	$1\frac{1}{2}, 1$	0.6, 0.67 cm
Late	$3\frac{1}{2}, 3$	3, 2	0.8, 0.75 cm
26	$3\frac{1}{2}, 3$	$3, 2\frac{1}{2}$	1.0, 0.95 cm
28	$3\frac{1}{2}$,	$3, 3\frac{1}{2}$	2.5, 3.6 cm
33	$5, 4\frac{1}{2}$	$4\frac{1}{2}, 3\frac{1}{2}$	6.5, 5.0 cm
37	7, 9	$7, 8\frac{1}{2}$	9.5, 12.5 cm
38	$5\frac{1}{2}, 7$	4, 6	6.5, 8.9 cm
39	$3\frac{1}{2}, 4$	2, 3	5.2, 6.5 cm
40	$2\frac{1}{2}, 1\frac{1}{2}$	3, 2	4.0, 3.2 cm
41	1	1	1.8 cm
42	..	..	1.3 cm
43	..	..	1.0 cm

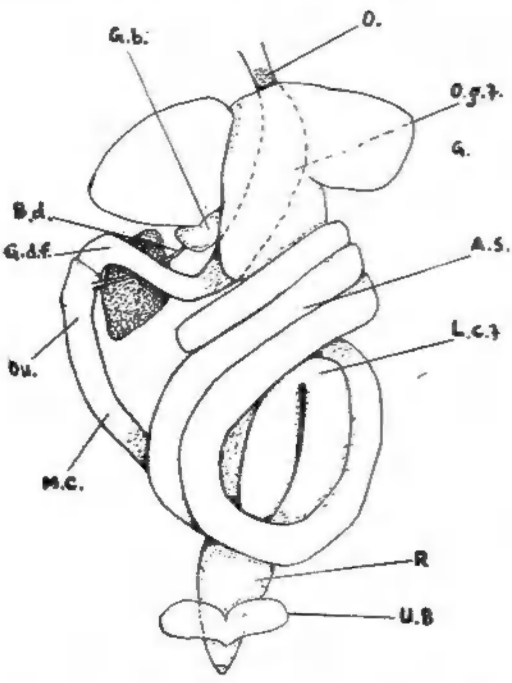

FIG. 12 Stage 40 x 70.

Fig. 12 Ventral view of digestive tract at Stage 40.

There is sudden reduction in the number and size of the coils, both of ascending and decending spirals (see Table No. 1). The feeding is stopped with the loss of beak and the denticles from the sucker (Stage 38).

There are two centers for the decrease in the length of spirals, independent for the ascending and descending spirals. The point for ascending spiral lies somewhere between *main coil* and "*loop of coil formation*". For the descending it lies somewhere beween the *loop of coil formation and the rectal coil.* The shortening part of both spiral does not show any external indication, in the form of fold or distortion of the surface. The regular pile-like displacement of the coils is disturbed, the course of the coils becomes confused. The shortening intestinal spiral brings drastic changes in the rest of the digestive tract.

The shortening in the gastric region observed during Stages 26-37 is carried on. The gastric region gradually acquires more sac-like dilated configuration. At first this region is running obliquely, rostro-caudad, along the left dorsal to right ventral side.

The stomach finding room due to the contraction of the coils, elongates obliquely towards posterior side, so that the *oesophageo-gastric flexion* is straightened up only slightly indicated by the bend between oesophagus and the cardiac end of the stomach. The gastric loop is also slightly indicated by the slight curve of the stomach along its rostral surface. Due to the caudad elongation of the pyloric end, the right part of the main coil is drawn round the *gastro-duodenal flexion*, thus forming a broad "U" with the stomach. The position of the *gastro-duodenal flexion* is taken by the distal end of the duodenum, thus forming the *duodeno-iliac flexion.*

The pancreas is also drawn up with the duodenum and occupies the space between the stomach and duodenum. The hepato-pancreatic duct opens in the middle part of the duodenum (Stage 40).

The liver lobes grow caudad and become thick and broad. The gall bladder comes

to lie along the ventral posterior end of the right lobe of the liver.

The rectal coil with the cloacal tube is contracted and forms a short pear-shaped broad rectum, which progressively decreases in diameter distally to form tubular cloaca.

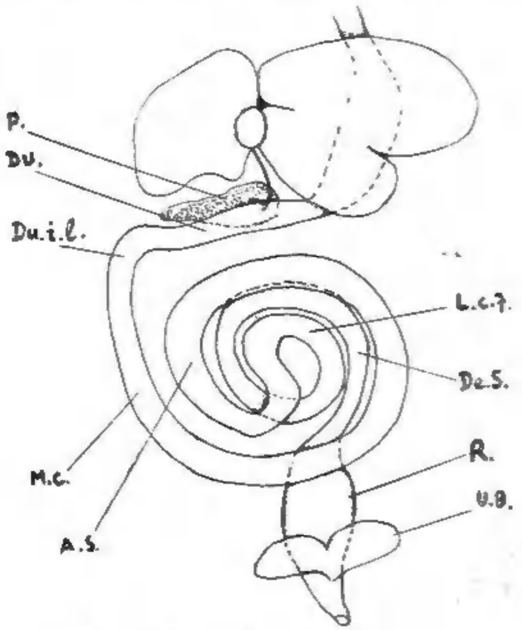

FIG.13 Stage 41 ×70

Fig. 13. Ventral view of digestive tract at Stage 41.

The transverse marking along the surface of the rectal coil and the anal tube disappear. A bilobed diverticulum makes its appearance at the ventral side of the cloacal region to form the urinary bladder, it also receives the ureters. The cloaca opens through a small opening lying along the posterior ventral side between the disappearing tail and the posterior end of the body, as the anal tube disappears (Stage 40).

Stage 41 (Fig 13) The duodenum is straightened up, horizontally. This phenomenon may be explained due to the shortening process in the main coil, it also drags intestinal spiral mesially. *Duodeno-iliac flexion* is quite marked.

The intestine is considerably shortened. Each spiral consists of one loose coil, with the persisting "*loop of coil formation*" in between.

Rectum shows a great decrease in size with the loss of tubular appearance. At Stage 40 it becomes pear-shaped with rostral broad part and caudad tubular part opening at cloacal aperture. The cloacal tube at this stage is shortened. The urinary bladder is well developed.

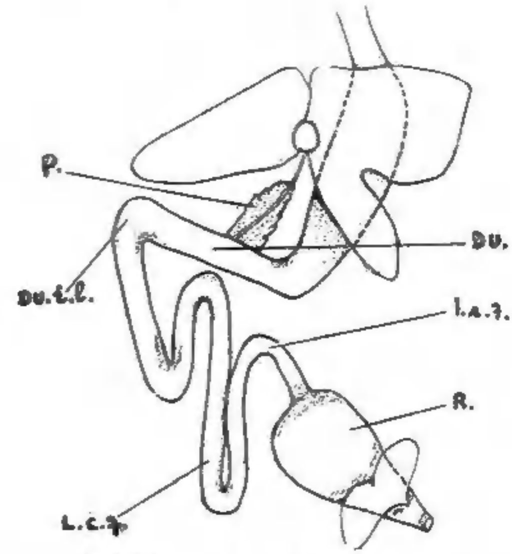

FIG.14 Stage 42 ×70.

Fig. 14. Ventral view of digestive tract at Stage 42.

The liver has massive and broad lobes. The gall bladder is roundish and is situated between the right and left main lobes of liver. Pancreas lies in the *gastro-duodenal loop*.

Stage 42 (Fig 14) The stomach shows regional differentiations.

(*i*) *Cardiac part*: It is the anterior broadest part of the stomach, lying along the dorsal side of the liver. It forms the slight *oesophageo-gastric flexion.*

(*ii*) *Main gastric part:* It is the middle part of the stomach, continuous with the cardiac part and has the same diameter. Shows the slight flexion (the gastric loop).

(*iii*) *Pyloric part:* It is the most posterior narrow part. The gastric part tapers

caudad to become continuous with it. It narrows down caudad to continue with the proximal part of the duodenum.

The intestinal spiral has disappeared, the ilium now consists of three well marked loops throughout its length.

(*i*) *Duodeno-iliac loop*, proximally continuous with the duodenum.

(*ii*) *Loop of coil formation:* lying between the duodenoiliac loop and the caudad loop. It is broadly gyred with the both limbs separated from each other.

(*iii*) *Caudal loop*: The posterior most loop. After this loop the distal part of ilium becomes continuous with the pear shaped rectum. The cloacal aperture becomes quite small and is guarded by the sphincter muscles.

Due to the straightening up of the coil the duodenum is slightly flexed rostrally. All three loops are held together by thin mesentery, which becomes quite demarcated at this stage.

Pancreas runs parallel to the bile duct.

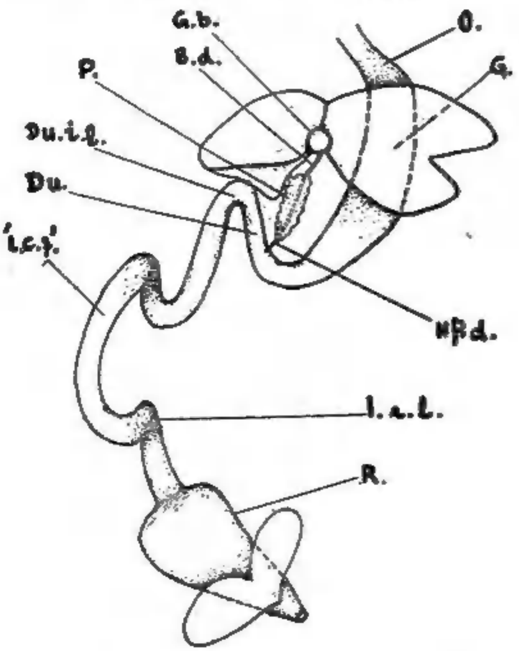

FIG. 15 Stage 43 ×70

Fig. 15. Ventral view of digestive tract at Stage 43.

This stage marks the end of the intestinal metamorphosis. The digestive tract attains the configuration of adult digestive tract. The stomach shows no change over its position in the last stage.

There is an enlargement in the gyre of intestinal loop. Due to the accentuation in the gyre of the intestinal loop the *duodeno-*

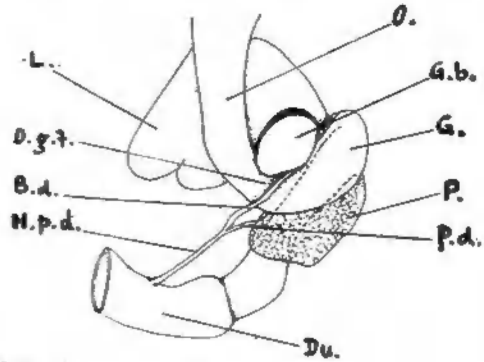

FIG. 16 Stage 38 ×60

Fig. 16. The gastric region and hepatopancreatic duct from dorsal side (intestinal spiral removed) Stage 38.

iliac loop is pushed rostrally, so that the duodenum forms a considerably narrow "U" shaped *gastro-duodenal loop*.

The mouth is fully gaped. The toadlet begins to feed upon small insects. The stomach of the toadlet is filled with small insects, predominately the ants.

Summary of Morphogenesis

The first noticeable change in the unorganised endodermal cellular mass, is the appearance of the gastro-duodenal and the dorso-ventral grooves, (Stage 21, Fig., 1 & 2) marking the initiation of the process of differentiation of digestive tract. These grooves manifest two opposite centers of growth, one acting along rostral left side, line of action being from right to left along horizontal plane—the ventral center of growth; the other acting along caudal right side, line of action being from right ventral to left dorsal side along oblique plane—the dorsal center of growth. They

cut off the anterior gastro-duodenal limb and the posterior anal tube, respectively (Figs. 3, 4 & 5). The center of growth acting at the gastroduodenal groove shifts towards the right ventral side of the main lobe (main coil), while the center of growth acting along the dorso-ventral groove lies on the right dorsal side of main lobe. So these centers come to lie opposite to each other (Fig. 6). The endodermal mass between these two opposite centers is shaped into a closed "U" shaped loop—"*the loop of coil formation*", marking the point for the formation of coils.

At Stage 24, the first coil of the both spirals is formed and the larval and the pre-larval developing digestive tract is in equilibrium, thus marking the "critical stage". The process of coil formation lasts until Stage 37 (see Table No. 1). The most frequent number of coils in both spirals at this stage is 7 to 9, Balinsky (1956) records 7 to 12 coils, just before metamorphosis, in *Xenopus laevis*. Feeding is stopped and the larva becomes sluggish. The sucker is gradually lost. Resorption of tail starts. Stage 38 marks the initiation of metamorphosis of digestive tube. At Stage 40 ascending spiral has two and a half coil while descending has three coils (Fig. 12). The shortening of the digestive tract is quite rapid. Through the next Stages 41, 42 & 43 it is completed. The Stage 43 has fully metamorphosed digestive tract, the feeding takes place at this stage. The metamorphosis of digestive tract is accompanied by the metamorphosis of tail.

Discussions

The modelling forces of the endodermal mass, in a tubular spiral form, are the ventral and dorsal centers of growth, acting in opposite directions. Similar conclusions are drawn by Albert (unpublished), Dalcq and Halter (1943), Kemp (1951). Albert has also experimentally proved that the formation of the grooves manifesting these forces, is due to the equilibrium of the coelomic fluid.

It has also been observed that the "loop of coil formation" is a stationary point not changing its position along the coils, persisting even after metamorphosis as an intestinal loop. Same conclusions have been drawn by Albert, and Dalcq & Halter (1943).

C. Bondi (1959) relates the morphogenesis of digestive tract with the contractions of embryo. In *Bufo melanostictus* it has been observed that the initiation of the spontaneous contractions takes place at Stage 17 and the endodermic grooves appear at Stage 21. The seat of contraction, at Stage 17, lies between the head and the trunk of the embryo, it shifts partially in the tail region at Stage 21. It is quite possible that the mechanical action of the opposite flexions of the tail and the endodermic mass may have an effect on the endodermal mass to produce the gastro-duodenal groove and the dorso-ventral groove. These later on model the endodermal mass in "S" shaped tubular configuration* (Stage 23, Fig. 5). The anterior and posterior lateral flexions of "S" allow free slight lateral bends of the endodermal mass. This state of affairs continues until State 24 when the anterior bend is blended by the formation of first coil of the ascending spiral (Fig. 7). This results in complete shifting of the center of movements at the base of tail and the anal tube. Hence there seems a definite co-relation between the modelling of pre-larval digestive tract and the movements of the embryo. A similar co-relation is seen at the time of metamorphosis. As the metamorphosis of the digestive tract starts (Stage 38) there are signs of the shortening and dwindling off of the tail. The digestive tract is fully metamorphosed at Stage 43 and there is a corresponding complete loss of the tail. During this period movements are taken up by the limbs. Bondi (1959) concludes similarly from his experiments. Berrill (1961) assigns importance to the stationary stages of certain invertebrate larvae, as it marks the initiation of the process of metamorphosis.

Quantity of food available also affects the development of the larval digestive tract. The culture of tadpoles, normally nourished on the vegetative food, shows normal development of both coils, while a poorly fed culture shows a deformed and

unhealthy digestive tract. Babk (1903, 1906) assigns this fact to the mechanical action of food on the development of the digestive tract. However in *Bufo melanostictus* it has been found that the feeding starts at Stage 25. This stage marks the rapid growth of digestive tract. Through the next stages (up to Stage 37) the larva is vigourously feeding and there is a very rapid elongation in the intestine (see Table No. 1) Stage 38 marks the initiation of the metamorphosis, the feeding is altogether stopped, tadpole becomes sluggish and there is a great reduction in the size of the intestine. Hence there seems to be a definite relation between the mechanical action of food, taken in, and the elongation of the intestine. Albert (unpublished) found similar sudden change at the advent of feeding in *Rana dalmatina*. However, the quality of food *i.e.*, herbivorous, carnivorous or mixed, does affect the length of the digestive tube, (Young, 1904). This is probably why the tadpoles of *Bufo melanostictus* reared in the laboratory differ in the development of their digestive apparatus, from those collected from nature, belonging to the same stage. In nature often the dead tadpoles and other dead organisms as earthworms, drowned crickets, cockroaches are fed upon by the tadpoles. Mosquito larvae, fairy shrimps and other arthropodes may also form the food of tadpoles (Bragg 1962). Thus nature provides mixed food to the tadpoles. It has already been pointed out, even the tadpoles of the same hatch may differ from each other. This phenomenon of heterochrony is universal during larval developmental stages (Balinsky, 1966).

To sum up, the development of larval and pre-larval intestine is affected by the

(*i*) larval movements.

(*ii*) quantity of food available.

(*iii*) quality of food available.

Stage 24 in *Bufo melanostictus* marks the end of embryonic period and start of larval period. At this stage the larva possesses following characteristics:—

——The length of tail almost equals the length of body.

——The gills almost disappear under operculum and the respiration is by internal gills.

——Eyes almost fully formed and effective.

——Feeding apparatus (suckers) sufficiently developed with all its constituents.

——Cement gland disappears or tends to disappear.

——Larva leads, from hence on, an autonomous active life.

——Movements of larva quick and autonomous, the center of movements mainly the tail.

——Digestive tract almost working with all the regions distinguished.

——Distinction between the trunk and the tail quite marked.

——No external evidence of fore and hind limbs. From hence on.

——The tail increases rapidly in size.

——Respiration by internal gills, and by lungs in later stages.

——Slight up and downward movements of the eyes.

——Sucker acts as a feeding apparatus and supports the tadpole during its attachment.

——Larva becomes very active, movements by the sideway lashings of the tail.

——Tadpole vigorously feeds and the digestive tract is very active.

——Hind limbs and fore limbs make their appearance. Similar critical stages have been determined in *Rana dalmatina* (Cambar & Marrot, 1954), *Alytes obstetricians* (Cambar & Martin, 1959) and (Cambar, 1959).

It has been pointed earlier that at this stage the prototype of future digestive tract is set up. During this period of pre-larval growth the overall development of the digestive tract has been rapid. Albert (op. cit.) records the rapidity of growth at this stage in *Rana dalmatina* also.

During the pre-metamorphic morphogenesis most active center of growth is the "loop of coil formation". This point lays the new coils, firstly, at the expense of

endodermal material from the thick main coil and the anal tube (Stages 23, Fig. 5 & 6). Then the material is provided by the reduction of diameter and the thickness of the already laid coils (Stage 33-37, Figs. 10& 11). Albert (op. cit.) records two most active centers of growth. One at the level of the gastro-duodenum, and the other "the point of retrogression". In *B. melanostictus* the prelarval center of growth is the "loop of coil formations". During metamorphosis there are three points of activity, gastro duodenum, "the loop of coil formation" and the rectal coil and anal tube. The "loop of coil formation" (summit of the coil,' Bataillion, 1891) which now acts as the center of reduction of the coils. The gastro-duodenum and the rectal and anal tube are active in the formation of stomach and rectum.

The development of stomach and duodenum is quite interesting. It implies the two phenomena, the growth and contraction. During pre-metamorphosis (Stage 37, Fig. 11) the gastric region is long and tubular "S" shaped, forming the gastric loop and the gastro-duodenal flexion. With the start of metamorphosis the length of this region is shortened and the stomach contracts so that it comes to lie obliquely in the left part of abdomen. (Stage 40, Fig. 12). This contraction drags the duodenum along it forming an inverted "V" shaped configuration, with a horizontal limb and avertical (caudad directed) limb, (Stages 41, 42, 43, Fig. 13, 14, 15). The gastro-duodenal flexion is now formed by the duodenum (=duodeno-iliac flexion), the gastric loop is quite reduced forming a very slight bend between the cardiac and pyloric ends of the stomach. A similar contraction of the rectal region also takes place with the widening and shortening of the rectum.

The nature of shortening process of the intestine is controversial. Reuter (1900) and Duesberg (1906) in *Alytes obstetricans* and *Rana fusca* regard it to be due to a "peristalsis, moving carinio-caudal, but permanent". Bower (1909), however, finds no permanent peristalsis during his studies on *Bufo lentiginosus*, by the help of X-Rays. She assigns the process of contraction to the mesentery. But the process of shortening and contraction seems to be intrinsic rather than extrinsic induced by certain hormones (Allen, 1938, Bytinsky-Salz, 1935). Earlier workers Ratner (1891), Young (1904) and Babk (1905) hold that the atrophy of digestive tract is due to stoppage of feeding. But Janes (1934), Bower (1909) and many other later workers have found it to be due to the histolysis of the intestinal tissues. In *B. melanostictus* there is no apparent cause of the shortening of the intestine except it is preceded by the stoppage of feeding. However, the shortening of digestive tube is accompanied by the shortening of mesentery. As already pointed out the shortening is not "carinio-caudal" but it is localized in definite centers along the digestive tract.

To sum up the morphogenesis of the digestive tract of *Bufo melanostictus* in few words, the complexity of the factors modelling the endodermal mass into the regionally differentiated larval spirally entwined digestive tract, then into a fully metamorphosed digestive canal; the succession of period of unequal gradual forces coming into play during this period, make this process very complex and heterogenous. These changes though drastic, well afford a stage-by-stage study.

Summary

(*i*) Normal development of digestive tract of *Bufo melanostictus* Schneider, has been studied, from undifferentiated endodermal mass to the stage when a definite adult digestive tract has been acquired.

(*ii*) Stage-by-stage study of morphogenetic changes are carried on by the dissection of the stages. Stage criteria employed is that suggested by Khan (1965).

(*iii*) The morphogenesis of the digestive tract of *Bufo melanostictus* could be divided into three periods, pre-larval, larval and post-larval.

(*iv*) Stage 24 marks an equilibrium between the pre-larval and larval development of the digestive tract, thus marking the "critical stage".

(*v*) The morphogenesis of digestive tract in *Bufo melanostictus* resembles with the morphogenesis of digestive tracts of other anura in essential respects.

LIST OF ABBREVIATIONS

A.S.	...	Ascending spiral.
A.t.	...	Anal tube.
B.d.	...	Bile duct.
C.g.	...	Dorso-ventral or caudal groove.
C.l.	...	Caudal loop.
De.S.	...	Descending spiral.
Du.	...	Duodenum.
Du. i. l.	...	Duodeno-iliac loop.
G.	...	Stomach or gastric region.
G.b.	...	Gall bladder.
G.d.f.	...	Gastro-duodenal flexion.
G.d.g.	...	Gastro-duodenal groove.
H.p.d.	...	Hepato-pancreatic duct.
I.r.f.	...	Ilio-rectal flexion.
L.	...	Liver.
L.c.f.	...	Loop of coil formation.
m.c.	...	Main coil or main lobe.
O.	...	Oesophagus.
O.g.f.	...	Oesophagio-gastric flexion.
P.	...	Pancreas.
P.d.	...	Pancreatic duct.
R.	...	Rectum.
U.b.	...	Urinary bladder.
Y.	...	Endodermal mass.

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